Clinical and Immunological Profile of Cryoglobulinaemia in North India*

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SUMMARY **India being primarily a tropical or sub-tropical country, the clinical problems aggravated by cold weather remain largely unexplored among Indian patients. Therefore, the present study was aimed at screening for the presence of cryoglobulins in clinical situations where their prescence would be expected.**

Seventy-eight patients and 15 healthy controls were studied in-depth. Cryoglobulins were detected in 37.1 per cent of the patients but in none of the controls. Of those patients in which cryoglobulins were detected, only 41.3 per cent had symptoms attributable to cryoglobulins while 58.7 per cent were symptomless. Type III cryoglobulins were encountered most frequently and rheumatoid factor was the most common antibody detected; the latter's presence always indicated the 'mixed' nature of cryoglobulins.

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Cryoglobulinaemia is a condition characterised by the presence of an unusual serum protein which can undergo reversible precipitation below normal body temperatures (below 37°C).[1,2] It is known to occur in a diverse group of diseases.[3,4] Cryoglobulins may produce clinical problems by two distinct mechanisms. Firstly, their precipitation in the circulation may lead to capillary blockage and compromised tissue perfusion.[5] Secondly, cryoglobulins often represent pathological circulating immune complexes.[6] Thus, they may also cause immune complex-mediated immuno-inflammatory tissue damage.

In the North Indian plain the ambient temperature falls to about 0-10°C between December and March. Such weather would produce cold-induced symptoms in patients with cryoglobulinaemia.[7] However, a systematic and detailed study of cryoglobulinaemia in India is not available.

The present study was aimed at screening North Indian patients suspected of having cryoglobulinaemia and evaluating the degree of symptoms in these patients which could be directly attributable to cryoglobulins along with the immunochemical characterisation of the cryoglobulins.

MATERIALS AND METHODS

Patient selection

Cryoproteins were screened in two categories of patients. The first category included patients with clinical features usually associated with cryoglobulinaemia[4,7] e.g. Raynaud's phenomenon, cold urticaria, dependent purpura, distal gangrene, arthralgia or arthritis, paraesthesias and glomerulonephritis, in any combination. The second category comprised those patients in whom the clinical features of cryoglobulinaemia were usually not present but in whom their diseases are known to be associated or suspected of being associated with cryoproteinaemia[2-4,8] e.g. (i) immunoproliferative disorders such as multiple myeloma, macroglobulinaemia and lymphoma; (ii) systemic collagen-vascular diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and progressive systemic sclerosis (PSS); (iii) chronic infections such as subacute bacterial endocarditis (SABE); and (iv) malignancies such as breast carcinoma and urinary bladder carcinoma.

Base-line work-up

A detailed history and physical examination was carried out in each

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patient with special attention to features suggestive of cryoglobulinaemia mentioned above. Routine laboratory investigations included complete blood count, erythrocyte sedimentation rate, routine urinalysis including microscopic examination, blood urea and serum creatinine levels.

In addition to these routine investigations, specific investigative procedures were carried out in each case for establishing the basic diagnosis. Thus, appropriate radiological investigations, bone marrow examination, serum electrophoresis, and urine test for Bence Jones proteins were carried out in patients with suspected multiple myeloma and related disorders. Lymph node biopsies were carried out on patients with suspected lymphoma. Echocardiography and multiple blood cultures were done on suspected SABE patients. Antinuclear antibody, C-reactive protein, other auto-antibodies and rheumatoid factor tests, appropriate radiological investigations, barium contrast studies, skin biopsy, etc. were carried out in order to confirm systemic collagen vascular diseases.

For urinary bladder carcinoma, intravenous pyelogram, urinary cytology, cystoscopy and biopsy were done. Breast carcinoma was confirmed by aspiration cytology and metastasis was detected by liver function tests, radio-nuclear liver scan and skeletal survey.

Once the diagnosis was established, the screening for cryoproteins was carried out.

Screening for cryoproteins

This was done by standard technique[1] with minor modifications. Blood (15ml) was collected in a warm syringe and placed in a test tube and allowed to clot in a 37°C water bath for two hours. Serum was separated after centrifugation at 38-40°C and kept at 4°C in a graduated centrifuge tube with 0.01% sodium azide as a preservative. The serum was observed daily for up to 10 days. If a precipitate was observed, the serum was again warmed at 37°C. If the precipitate redissolved, it was considered confirmatory of cryoprecipitate and the patient was considered to have cryoproteins. The sample was again kept at 4°C and allowed to reprecipitate. The sample was then centrifuged at 4°C and the cryoprecipitate was separated. It was washed with cold saline and dissolved in a minimum amount of phosphate buffered saline (0.05 M, pH 7.2 at 37°C) and further analysis was carried out as follows:

Analysis of cryoprecipitate

1. Concentration of cryoprecipitate

The volume of serum kept for the detection of cryoproteins was noted. The cryoprecipitate formed was removed, washed and redissolved in a known amount of phosphate buffered saline. The concentration of protein in this sample was determined by the standard technique.[9] The total amount of protein in this whole suspension represented the total amount of cryoprotein in the volume of serum kept for observation and this was finally expressed as mg per ml.

2. Identification for the presence of IgG, IgM, IgA and C3 in cryoprecipitate

This was done by using two techniques:

a. By the standard double diffusion technique of Ouchterlony.[10] The redissolved cryoprecipitate was applied to a well which was cut out in agar gel in the centre of a culture dish and surrounded by wells containing anti-IgG, anti-IgA and anti-C3 sera (M/S Immunodiagnostics, Delhi, India). Each well was 2.5 mm in diameter and was separated from other wells 5 mm edge to edge.

b. By single radial diffusion technique.[11] Monospecific antisera against IgG, IgM and IgA were commercially obtained (M/S Immunodiagnostics.

3. Gel filtration of cryoprecipitate

Further analysis of the cryoproteins was carried out by gel-filtration on Sephadex G-200 column. The column was equilibrated with acetate buffer (0.1M, pH 4). Standard techniques of column chromatography were used.[12]

4. Analysis for immunoglobulins and light chains

Fractions so separated were analysed for IgG, IgM and IgA using single radial diffusion techniques[11] and pure fractions were pooled separately.

Typing for light chains was done by using anti-kappa and anti-lambda antisera (Cappel Lab, Cochranville, Pen., U.S.A.) by double diffusion in agar gel.

5. Screening for auto-antibodies in cryoprecipitate

Redissolved cryoprecipitate was screened for the presence of the following auto-antibodies:

Rheumatoid factor at room temperature and at 4°C, antinuclear antibodies (ANAB), smooth muscle antibody (SMA), antimitochondrial antibody (AMA), parietal cell antibody (PCA), microsomal antibody (TMA).

a. Rheumatoid factor was tested by using commercially available kits (Wellcome Lab, London, England) for the standard latex agglutination test. Positive and negative sera (supplied with the kits) were included in each batch of tests.

b. Other auto-antibodies, i.e. ANAB, SMA, AMA, PCA and TMA were detected by standard indirect immunofluorescence technique[13] as standardised earlier in this laboratory.[14] Commercially available fluorescein-labelled polyspecific anti-human IgG+M+A (M/S Immunodiagnostics, Delhi) was used in this study. TMA, if detected, was further titrated by passive haemagglutination technique using commercially available kits (M/S Fuzizoki, Japan).

6. Australia antigen

This was tested by standard counter-electrophoresis technique[15] using commercially available antibody against hepatitis B surface antigen (Wellcome Lab).

Analysis of serum

1. Total serum protein was estimated by the standard technique.[9]

2. Auto-antibodies including rheumatoid factor, ANAB, SMA, AMA, PCA and TMA were analysed in the same manner as in the cryoprecipitate (described earlier).

3. C3 levels in the serum were estimated by single radial immunodiffusion in 1.5% agarose[11] using commercially available monospecific antisera (M/S Immunodiagnostics, Delhi). Serum with a known amount of C3 level was used as a standard.

4. Australia antigen was detected by the standard counter-immunoelectrophoresis method[15] as in the case of cryoprecipitate.

RESULTS

The present study included a total of 78 patients and 15 normal healthy adults as controls. Table 1 gives the details of the patients studied and their status with regard to cryoglobulinaemia.

Trace amounts of cryoglobulins were present in different diseases. In many such cases, it was difficult to obtain adequate amounts of cryoproteins for detailed immunochemical analysis. However, in patients with SLE, PSS, multiple myeloma, SABE, breast cancer and essential mixed cryoglobulinaemia, the amount of cryoglobulins was adequate for further analysis. Results are given in Table 2. Type III cryoglobulins were the most common. The auto-antibody most often seen in cryoglobulins was rheumatoid factor. Besides immunoglobulins, C3 was also often seen in cryoproteins.

The analysis of the symptoms due to cryoglobulins in different groups of diseases varied markedly. Thus, among the 29 out of 78 patients who showed cryoglobulins in their sera, all of the three patients with essential mixed cryoglobulinaemia, three out of the five with SABE, one of the two with multiple myeloma, four out of the six with SLE and one of the two with PSS had clinical features attributable to cryoglobulins. In other words, this study showed that of the 37.1 per cent patients screened to be positive for cryoglobulins, only 41.3 per cent of them had symptoms attributable to cryoglobulins while the remaining 58.7 per cent were asymptomatic. Table 3 gives the common clinical features which could be attributed to cryoglobulinaemia in these patients.

DISCUSSION

Cryoglobulinaemia is a pathological state which produces a well-defined clinical syndrome.[1,3,5] Thus, irrespective of the type of cryoglobulin and whether the underlying cause is known (secondary cryoglobulinaemia) or unknown (essential, idiopathic), the clinical features of slugging, hyperviscosity, vasculitis of the skin and other organs, are common to all types of cryoglobulinaemia.[4] Therefore, it would appear that irrespective of its composition, the physical presence of cryoprecipitate in the circulation itself must be directly responsible for

Table 1 Cryoglobulinaemia in different diseases

Diagnosis	n	M – F	Median age (years)	Mean duration (months)	Cryoglobulin (mg/ml)	Type	No.
1. Systemic collagen diseases	30	3 – 27					10
SLE	10	0 – 10	31	24	trace to 0.80	III	6
RA	10	2 – 8	41	54	0		0
PSS	10	1 – 9	34	62	0.40 – 0.60	III	2
2. Multiple myeloma	10	7 – 3	45	4.6	4.60 – 6.00	I	2
3. Malignancies	25	13 – 12					11
non-Hodgkin's lymphoma	5	5 – 0	27	3.2	0.20	*	1
breast cancer	10	0 – 10	54	6.4	0.40	III	1
urinary bladder cancer	10	8 – 2	55	6.0	0.02 – 1.15	*	9
4. SABE	10	6 – 4	26	2.3	2.00 – 4.20	III	5
5. Essential mixed cryoglobulinaemia	3	1 – 2	43	18	0.15 – 3.20	II	3
6. Normal controls	15				0		0

*Amount of cryoprotein not sufficient for detailed immunochemical analysis (SLE = systemic lupus erythematosus, RA = rheumatoid arthritis, PSS = progressive systemic sclerosis, SABE = subacute bacterial endocarditis).

Table 2 Immunochemical analysis of cryoglobulins

Diseases	No of patients	Type	RF +ve	ANAB	Other auto Ab	C3	HBsAg
SLE	4	III	2	0	0	4	0
PSS	2	III	2	0	0	0	0
SABE	5	III	5	0	0	4	0
MM	2	I	0	0	0	0	0
BrCa	1	III	1	0	0	0	0
UrCa	4	?	4	0	0	3	0
EMC	3	II	3*	0	0	0	0

*In one patient only at 4°C.
(SLE = systemic lupus erythematosis, PSS = progressive systemic sclerosis, SABE = subacute bacterial endocarditis, MM = multiple myeloma, BrCa = breast carcinoma, UrCa = urinary bladder carcinoma, EMC = essential mixed cryoglobulinaemia.)

Table 3 Clinical features attributable to cryoglobulinaemia

Manifestations	No. of patients	General incidence (%)
Cutaneous		
Raynaud's phenomenon*	5	17.2
Vascular purpura	3	10.2
Gangrene	2	6.8
Cold urticaria	2	6.8
Livedo	0	0
Leg ulcers	0	0
Articular		
Arthralgia	10	34.4
Neurological		
Paraesthesia/numbness	6	20.6
*Renal involvement***	6	20.6
Haemorrhages	0	0
Abdominal pain	0	0

*Raynaud's phenomenon present in cases where cryoglobulins were absent are not included. This phenomenon in progressive systemic sclerosis may be due to vasospasm.
**Renal involvement in patients was considered on the basis of finding of albuminuria and/or haematuria microscopic or macroscopic and high blood urea. Kidney biopsy in two such patients showed membranoproliferative glomerulonephritis.

these clinical features. The additional clinical features may be related to the underlying diseases including collagen-vascular diseases, chronic infections or malignancies. In patients with essential mixed cryoglobulinaemia, the additional clinical features include dependent purpura, arthralgia, weakness, lymphadenopathy and hepatosplenomegaly.[16]

Because India is mainly a tropical or subtropical country, the winter is either nonexistent or very brief. Therefore, patients with cryoglobulinaemia may remain symptomless and undetected. Moreover, most physicians are not familiar with the clinical syndrome related to cryoglobulinaemia. If a patients develops the clinical features of cryoglobulinaemia during the short but sharp winter of the North Indian plains or the country's "hill-stations" he or she may go undiagnosed. The main purpose of this paper was to determine the extent of the problem of cryoglobulinaemia in India and to bring out the clinical features of this physico-chemical serum protein abnormality.

The present study brought out that if investigated in depth, cryoglobulinaemia is not uncommon. Thus, on screening patients with diseases in which cryoglobulinaemia is known to occur, 37.1 per cent were shown to have cryoglobulins in their serum. However, because the ambient temperature is not very low in this country, only 12 out of 78 (15.3%) showed clinical features attributable to cryoglobulins. (Table 3 gives these clinical features). It is important to know these manifestations because plasmapheresis is a definitive mode of treatment for problems related to cryoglobulins.[17]

In agreement with the other major studies on cryoglobulins,[3,4,8] type III cryoglobulinaemia was the most common in Indian patients. This work has further confirmed that the presence of rheumatoid factor or antinuclear antibody activity in cases with cryoproteins indicates the 'mixed' nature of these proteins.[18] Thus, if the facilities for in-depth analysis of cryoglobulins are not available, a simple screening test for RF and ANAB would be helpful in categorising the patient as either belonging to type II or III cryoglobulinaemia rather than the type I group for which these tests are negative.

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